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THE EFFECT OF CERTAIN DRUGS ON THE SOMATIC NEUROMUSCULAR MECHANISM.

John Randolph Murray, B.Sc.

A thesis submitted in conformity with the requirements for the degree of Master of Science by the University of Alberta.

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INTRODUCTION.

Before examining the action of drugs on the response of skeletal muscle to direct or indirect stimulation, it is essential to have a thorough knowledge of the anatomical, physiological and biochemical factors involved in neuromuscular phenomena and of the general pharmacological properties of the drugs investigated.

1. INNERVATION OF SKELETAL MUSCLE

Anterior horn cells of the spinal cord provide the nerve supply of skeletal muscles. These nerves extend by way of the ventral nerve root to peripheral nerves and on to the motor end plates of the muscle fibres. Each motor neuron may supply over one hundred muscle fibres. Cooper (1) has demonstrated the extensive branching using histological methods.

The normal rate of discharge of the somatic motor neuron in the anterior horn of the spinal cord is 5 - 10 per second, and this continues intermittently with other motor units of the same muscle. More frequent discharges are caused by increased stimuli and tetanus occurs at a rate of 60 - 100 per second. Muscle contraction can also be increased by the recruitment of other muscle fibres so that when all, or most of the motor units controlling a muscle are firing at a tetanic rate, a maximal contraction is obtained (2). Long sustained responses, such as those underlying the postural mechanism, are maintained by rates of discharge which are relatively low and which cause no fatigue.

2. MORPHOLOGY OF THE MOTOR END PLATE

It has been known for many years that the motor nerves to a skeletal muscle fibre end in an arborization at

a special region of the muscle known as the motor end plate. It has also been known that these efferent nerves may branch and re-branch many times. In the perimysium, further branching is evident after which the nerve fibres lose their myelin sheaths and pass to the individual muscle fibres.

The recent histological work of Couteaux (3) on the motor end plate has emphasized a structure which is separate from and outside of the terminal arborization of the nerve. Material which stains with the dye, Janus green, is arranged in palisades. These minute rod-like masses appear to be lined up side by side perpendicular to the surface of the nerve terminals. Fine thread-like projections extend from the ends of the rods that face the muscle into the surrounding sarcoplasm. Cross-sectional views of the end plates show that the nerve endings lie in gutters or grooves. The material which stains with Janus green lines these gutters on the muscle side.

This palisade arrangement has been demonstrated in the mouse, guinea pig and lizard. It is less well developed in the frog. Palisades have long been recognized in certain fish. For example, in the case of the electric organs of the Torpedo, the evidence indicates that the single unit, the electroplax, is like a neuromuscular end-plate, without the accompanying muscle fibre, with its conductile and contractile elements. These palisades line the region where the terminal arborization of the nerve makes contact with the electroplax. Since there is a striking resemblance between the palisades in these different situations, it would appear that this material is of

some physiological importance both in the electroplax and in the end plate.

The motor nerve plates show marked variation in size and form. Carey (4) has shown that relaxed muscle fibres with coarse, widely separated striations are associated with retracted nerve plates consisting of coarse fronds; whereas contracted muscle fibres with broad, compact bands are associated with expanded nerve plates consisting of thinner branches. He suggests that these variations are determined by functional ameboidism. When the functional activity is increased, the surface area of the nerve plate increases. This would appear to favour the conduction of impulses from nerve to muscle.

3. IMPULSE TRANSMISSION ACROSS SYNAPTIC AND NEUROMUSCULAR JUNCTIONS.

(a) CHEMICAL TRANSMISSION AT THE PERIPHERY

It is now generally accepted that the effects of impulses in the efferent post-ganglionic fibres of the autonomic nervous system are transmitted to involuntary muscle and gland cells by the liberation of two transmitters - adrenaline (sympathin) and acetylcholine (5). The nature of the transmitter also determines whether the effect produced by the arrival of nerve impulses at the endings will be augmentation or inhibition of an activity; and very often the two transmitters produce opposite effects on the same layer of involuntary muscle.

It has been suggested (5) that histamine may act as a third transmitter. The evidence, which is not yet nearly

as definite as for adrenalin and acetylcholine, indicates that histamine may be released by the so-called "antidromic" nerve impulses at the endings of terminal nerve branches supplying the network of minute blood vessels to the skin.

(b) TRANSMISSION AT GANGLIONIC AND NERVE-TO-END-PLATE SYNAPSES

Dale's theory (5) postulates chemical transmission at motor neuromuscular and ganglionic synapses as a process essentially different from that by which the excitatory process is conducted along either a nerve or a muscle fibre in continuity. Regarding the transmission of the influence of the nerve impulse to the end plate mechanism, he thinks that the momentary process of excitation at a point on a nerve or muscle fibre is due to a biochemical change, possibly the mobilization and reattachment of potassium ions, entailing a momentary loss or reversal of a resting surface potential at each successive point as the process is conducted in either direction along the fibre by the successive closure of local electrical circuits. When this electrically conducted process reaches the ending of the nerve fibre it is thought to release a tiny amount of acetylcholine which, during its momentary persistence, causes an excitatory depolarization at the surface of ganglion cell or motor end plate, by a mobilization of potassium ions; and that the process of excitation is then, as before, electrically propagated along the post-ganglionic nerve fibre or muscle fibre. It may be shown experimentally that when ganglion cells or motor end-plates are treated with curarine, they

no longer respond to application of acetylcholine or to nerve impulses at the synaptic endings but still respond normally to the application of a potassium salt.

There is considerable evidence (6,7,8) for a specialized function of the end-plate region. It has been shown, for example, in single muscle fibres that the end-plate region is depolarized by very small amounts of acetylcholine or nicotine or by somewhat larger amounts of caffeine (9,10) and that the rest of the surface of the muscle is a thousand or more times less sensitive to these substances. It is probable that many other substances which possess a stimulating action on skeletal muscle similar to that of acetylcholine or nicotine also act by depolarizing this specialized part of the muscle. There is evidence too, in the case of curare and other drugs which antagonize acetycholine and nicotine that these drugs also act at the end-plate by preventing its depolarization.

Two hypotheses have been proposed for the specialization of the end-plate. It has been suggested that the end-plate lacks a protective covering which is present elsewhere. This hypothesis has no experimental support.

The other hypothesis is that there is a chemically differentiated substance in this region which is absent elsewhere in the membranes of muscle and nerve. This is usually designated the receptive substance, after Langley. At present it is defined only in terms of its reactions in intact cells. It is therefore, highly hypothetical and must remain so until further data are obtained.

Acheson (11) puts forth the following questions re-

garding the parts of the structure of the neuromuscular synapse that give it its specialization. (a) Are the palisades the receptive substances? (b) Or do they represent the large amount of cholinesterase which has been demonstrated to be concentrated in this region? (c) Are they associated with some accessory physiological processes having to do with the end-plate potential?

Monnier (12) pointed out that there are many facts which are inconsistent with the theory of chemical mediators to skeletal muscle. Efforts to explain these inconsistencies have led many workers to concentrate on a non-chemical hypothesis of synaptic and neuromuscular transmission. Important in this field is the electrical hypothesis of Eccles as published in 1946 (13). Although this theory offered a satisfactory explanation of many fundamental observations on synaptic transmission, some of which were inexplicable in detail previously, it still encountered difficulties in explaining the action of curare and of anti-cholinesterases on synaptic transmission in ganglia and skeletal muscle. In 1948 Eccles stated that the electrical hypothesis could not be reconciled with more recent experimental results on neuromuscular transmission (14). Consequently he and his co-workers now believe that acetylcholine is the sole mechanism concerned in neuromuscular junction transmission. Concerning spinal cord transmission, Eccles was satisfied that the electrical theory provides a suitable explanation.

Monnier (12) has concluded that neither the chemical

nor the electrical theory alone can provide a satisfactory interpretation of all the experimental data. He suggests that sometimes both theories must be considered simultaneously and that by using such a dual theoretical interpretation a satisfactory picture may be obtained.

4. ACTIONS OF DRUGS USED IN THIS INVESTIGATION.

(a) ANTIHISTAMINE DRUGS - BENADRYL AND PYRIBENZAMINE.

Doses of antihistaminic drugs commonly employed in animals have little effect on respiration nor is there evidence that human therapeutic doses have any such effect. Following large oral or parenteral doses, respiratory stimulation is seen in unanesthetized animals. Such stimulation usually preceded or was concurrent with marked excitability, tremors and convulsions. Secondary respiratory depression may have been a contributory cause of death (15).

When Benadryl or Pyribenzamine is rapidly injected intravenously into dogs, a transient hypotension is induced. When the drugs are given slowly or by other routes, a slight hypertension of several minutes duration is produced (16). The exact mechanism of these actions has not been determined but it has been suggested (17) that the hypertension caused by Pyribenzamine was in part due to a stimulation of the central nervous system. Page and Green (34) have shown that Benadryl produces refractoriness of blood vessels to chemical stimuli. This appears to be a direct action on the blood vessels. When vascular refractoriness is elicited, the capacity

of the vascular tree appears to be increased. When Benadryl is perfused along with chemical stimulants through the vessels of isolated rabbits' ears, vasoconstriction is significantly reduced or abolished. That the blood vessels were not irreversibly poisoned by the drug was shown by a return to normal of the response when the Benadryl was washed out.

Most antihistamine drugs exert some local anaesthetic action. Graham (18) found that Benadryl is 2.5 times as potent as procaine. He suggested that there is a relationship between the potency of the drug as a local anaesthetic and its potency as an antihistamine. Reuse (19), however, suggests that the relation is between the anti-acetylcholine and the local anaesthetic effects of the drugs. Leavitt and Code (20) have shown that the local effect of the drugs wears off in approximately an hour, and that after that time comparative antihistamine studies can be carried out.

Several antihistaminic drugs, including Benadryl and Pyribenzamine have been shown to potentiate the action of adrenaline (21, 22, 23,). This effect has been demonstrated only in biological preparations and not in the unanaesthetized animal or man. More recent drugs described by Achenbach and Loew (24) antagonize the action of adrenaline. Since some potent antihistamines potentiate and others antagonize adrenaline, it is now generally considered that no relationship exists between their adrenaline and antihistamine effects (25).

Some autonomic effects of Benadryl and Pyribenzamine

have been reported by Dreyer (26) who has shown a diminution of salivary flow on chorda tympani stimulation in cats after the intravenous injection of 1.5 mg/Kg. of Benadryl. This diminution did not occur on cervical sympathetic ganglion stimulation. The former was completely blocked with a dose of 5 mg./Kg. but the latter only partially blocked. Motor response to vagal stimulation also was partially blocked.

Pyribenzamine has been found by Younkman, et al. (22, 27) to block the salivary secretogogue effect of histamine but to have no effect on salivation produced by pilocarpine, epinephrine, or stimulation of the chorda tympani or the cervical sympathetic ganglion. Further, this drug potentiated the effect of epinephrine on the nictating membrane of the cat. It is doubtful whether the antihistamine and anti-allergic activity of Benadryl is closely related to atropine-like action since such action is weaker than that exerted by such antispasmodic drugs as Pavatrine and Trasentin which do not prevent anaphylaxis or effects of histamine on bronchioles and the vascular system (21, 28). According to Loew (25) the atropine-like action of antihistamine drugs is unimportant from a clinical standpoint since these drugs seldom produce tachycardia or inhibit salivation. He points out, too, that from a theoretical standpoint, even a slight degree of atropine-like activity could be important if the drugs simultaneously enhanced activity of tissues receiving sympathetic innervation and that consideration must,therefore, be given to evidence that antihistamine drugs potentiate responses to epinephrine and stimulation of adrenergic nerves.

The failure of Benadryl to elevate the blood sugar level in man or to decrease glucose tolerance (29,30) strongly suggests that no appreciable sympathomimetic action was exerted since a sensitive indicator of epinephrine action is a rise in blood sugar (31). Loew (25) was unable to demonstrate any synergism between ephedrine and Benadryl in reducing mortality of guinea pigs upon induction of histamine shock. He suggests that a strong argument against the hypothesis that sympathomimetic properties, or some type of adrenergic potentiation, is a requirement for potent antihistamine action is found in the fact that a compound somewhat closely related to Benadryl antagonizes histamine as well as certain effects of epinephrine (32,33); also there is a recent demonstration (24) that certain adrenergic blocking agents are also exceedingly potent antagonists of histamine, both actions being rapid in onset and of remarkable duration.

Benadryl has been shown (23) to have a slight degree of antispasmodic action on the dogs' intestine but not on the uterus, whereas Pyribenzamine is capable of inducing spasm of intestinal and uterine muscle of animals in vivo and in vitro. Both drugs diminished the spasmogenic action of histamine on the duodenum and uterus.

It is generally believed that histamine antagonists act by receptor competition. The theory is that they become fixed, in varying degree and with varying firmness, to receptors of the tissues on which histamine acts, and, by this preferential fixation or competition, partially exclude or block

the access of histamine to these receptors (35). Dale (36) distinguishes between histamine which is released by the cell on which it reacts and that which when released acts only upon more remote structures. The former, he calls intrinsic and the latter, extrinsic histamine. In man, the antihistamine drugs are usually much less effective against intrinsic than against extrinsic actions of endogenous histamine. For example, in bronchial asthma, where the histamine is liberated by the reacting structure, these drugs have little effect but show a high degree of antihistamine activity in chronic urticaria where extrinsic histamine is involved.

In a study of the toxicity of Benadryl in various animals, Rieveschl and Gruhzit (37) reported that lethal doses by all routes produced violent excitement, convulsions and respiratory failure in a short time. They associated the death of the animals primarily with respiratory failure.

Graham (38) reported that with usually sub-lethal doses of Benadryl, when death occurred it seemed to be a result of general exhaustion rather than from involvement of any specific organ system. It has also been suggested that toxic doses of Benadryl cause death by respiratory and cardiac failure (39). A direct comparison (23) of the acute toxicity following intraperitoneal injection in mice of Pyribenzamine, Benadryl and a third antihistamine drug revealed no important difference in the values for LD50. Loew reports (25) that none of the antihistamine drugs are highly toxic and that it is unlikely

that any major differences exist in relation to the acute toxicity of the various compounds. Toxic doses of several antihistaminics, including Benadryl and Pyribenzamine, in several animal species induced tremors, then convulsions which were sometimes followed by depression and finally death. Loew says that death appeared to be due to cardio-respiratory depression.

Macht and Hoffmaster (41) reported on the influence of Benadryl and Pyribenzamine on the neuromuscular system of rats. Three sorts of experiments were performed:

(1) on the behaviour of rats in a circular maze giving data on neuromuscular activity and on discrimination or "choice" reactions:

(2) On the coordination of rats running across a tightly stretched horizontal rope:

(3) On neuromuscular work performed by rats climbing a vertical rope.

Doses of both drugs ranged from 0.1mg. to 2.0mg. given intraperitoneally or subcutaneously to rats weighing 150-200 gm. The results of their experiments pointed to the central nervous system being more affected than the muscular responses.

(b) CAMPHOR

Camphor stimulates the central nervous system. In mammals this action is more marked on the higher centres although all portions of the cerebrospinal axis share it to some degree. In high doses the drug produces epileptiform

convulsions which are largely cortical in origin.

The effects of non-convulsive doses of camphor on the respiration may result. The greater part of this effect is probably a reflex stimulation from the pain caused by the irritating subcutaneous injection of camphor.

(c) NIKETHAMIDE (ANACARDONE, CORAMINE)

Nikethamide acts as an analeptic. It counteracts the anaesthetic action of a number of the central nervous system depressants. Nikethamide is less effective in opposing barbiturate depression than is picrotoxin or metrazol (40). In toxic doses nikethamide causes convulsions from excitation of higher motor centres.

Nikethamide is a respiratory stimulant and apparently exerts this effect in doses which have little action on other portions of the cerebrospinal axis.

(d) CAFFEINE

(1) Central Action

Caffeine is a powerful central nervous system stimulant. The main sites of stimulation are the cortex, medulla and spinal cord. The cortex is first affected, next the medulla and the cord is stimulated only by very large amounts.

Caffeine stimulates all portions of the cortex, but its main action is on psychic and sensory function.

Caffeine acts on the respiratory, vasomotor and vagal centres in the medulla when large doses are used and these actions are most clearly seen when the medulla has been previously de-

pressed.

After the administration of large amounts of caffeine the entire central nervous system is stimulated, including the spinal cord. Reflex excitability is increased. Excitation of the lower motor centres may lead to clonic convulsions.

(2) On Smooth Muscle

Caffeine tends to dilate blood vessels by a direct effect on vascular musculature but the tendency is opposed by stimulation of the medullary vasomotor centre. Dilute solutions increase, and high concentrations depress, the tone and amplitude of contractions of isolated intestinal strips.

(3) On Voluntary Muscle

The effect of caffeine on voluntary muscle is disputed. Caffeine allays fatigue, which some investigators attribute to a direct action on the muscles and others to a central masking of the recognition of fatigue. Certain species of frogs show marked rigor following the injection of caffeine. The action is peripheral rather than central in origin for it is not influenced by nerve section or curare. A definite rigor can be produced in mammalian muscle by injecting caffeine directly into a muscle. An isolated nerve-muscle preparation is less susceptible to fatigue if a low concentration of a xanthine is present in the bath fluid.

STATEMENT OF THE PROBLEM.

STATEMENT OF THE PROBLEM

Studies of the antihistamine drugs have shown that these compounds, in addition to their histamine-blocking activity, have, in varying degree, anti-acetylcholine, local anaesthetic, antispasmodic, sympathomimetic or sympatholytic actions which appear to be unrelated to their antihistamine effects. The central nervous system is also affected in many instances: in man, the principal effect is sleepiness; in animals, convulsions and excitement often result from high doses.

Since the chemical mechanism of transmission in the somatic junction is analogous to that in parts of the autonomic system, and possibly to that in the central nervous system, it would follow that the antihistaminics might also affect peripheral somatic endings. One purpose of this investigation is to examine the effects of certain antihistamine drugs on the somatic neuromuscular mechanism.

As certain central nervous system stimulants have been shown to have effects on striated muscle (e.g. caffeine) and on the somatic neuromyal junction (44) it was also thought to be of interest to examine the peripheral effects of certain analeptics.

EXPERIMENTAL

1. Method

1. EXPERIMENTS ON THE GASTROCNEMIUS MUSCLE OF THE RAT.

A. Apparatus (Figure 1.)

(i) Method of Stimulation.

Various forms of stimuli, such as mechanical, thermal, chemical and electrical, applied to the muscle directly or to the innervating nerves, are used in experimental work.

Electricity was used throughout as it is available and convenient, easily controlled as to strength and speed of application and least destructive of the tissues stimulated.

Induced current regulated by an inductorium was used as the electrical stimulus. When a muscle or nerve is stimulated by the current from an inductorium two responses occur; first, when the primary circuit is closed, and second, when it is broken. The resulting electrical shocks are termed the make and break shock respectively, and ordinarily one or the other is rendered ineffective by means of a shut switch which shorts the secondary coil of the inductorium at the time when the undesired shock is produced. When it becomes necessary to administer single shocks repeatedly at regular intervals over a period of time, some automatic device is desirable.

The apparatus used was as follows; two fibre disks were mounted on the shaft of a motor turning at a speed of one revolution per minute. Slots were cut in the periphery of the disks in such a manner that as the vanes revolve they depress two long steel springs from a Harvard Vibrating Interrupter which close mercury contacts. The two disks are not in exact alignment, which results in the outer mercury contact

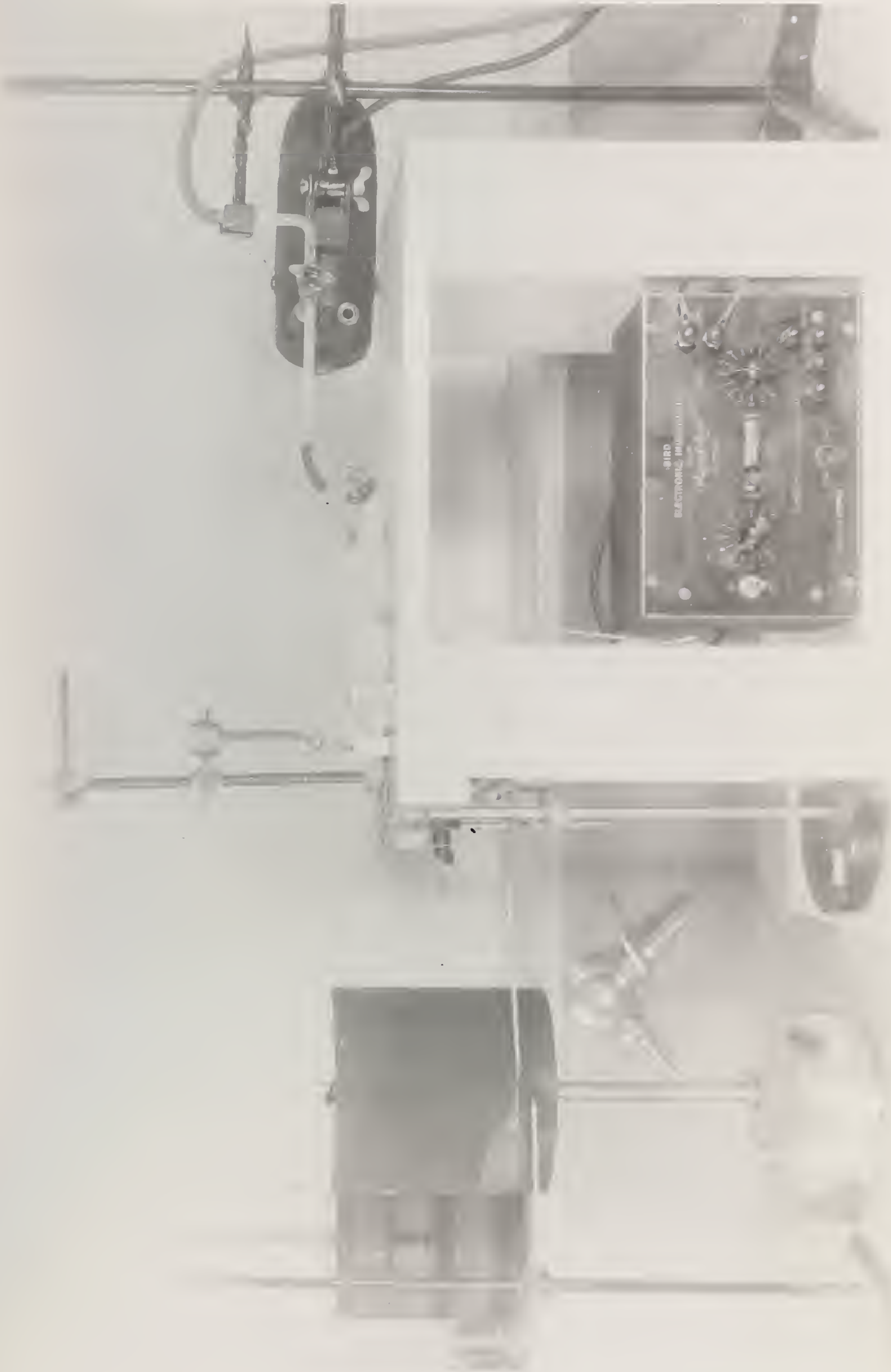


FIGURE 1

being closed before the inner set. Since this contact is connected across the secondary of the inductorium, the secondary is shorted, and as the disks continue to turn, the primary circuit is closed; after which the secondary circuit is opened by the outer disk to be followed by the production of a break shock as the primary circuit is opened by the inner disk.

Six vanes on the disk produce stimuli at intervals of ten seconds. A current of two volts from a storage cell supplies the primary current.

It is necessary to have the motor of the automatic device some distance from the inductorium to prevent it setting up induced currents in the inductorium.

This method did not prove entirely satisfactory in this work and was replaced by an electronic stimulator (Fig. 1.).

A 110 volt A.C. current charges, through a resistance, a condenser which, when fully charged discharges a direct current through a second resistance to give a single shock. It is possible to adjust the apparatus so that shocks are delivered at definite intervals. The first resistance in the circuit requires a different time interval for the condenser to become fully charged. The second resistance can be adjusted to deliver any degree of shock over a limited range.

In all this work shocks which produced maximal response were used at ten second intervals. This method of stimulation proved highly satisfactory.

(ii) Method of Securing Animals and Recording.

Albino strain rats were used throughout. The rat is immobilized on its back by passing cords attached to three

legs through holes in a small table. The other hind leg was rigidly clamped at the knee joint and the ankle bone in clamps which are fixed to the table. A cord attached to the calcaneous tendon was led over a pulley and attached to an isometric spring muscle lever which recorded on a slowly moving kymograph.

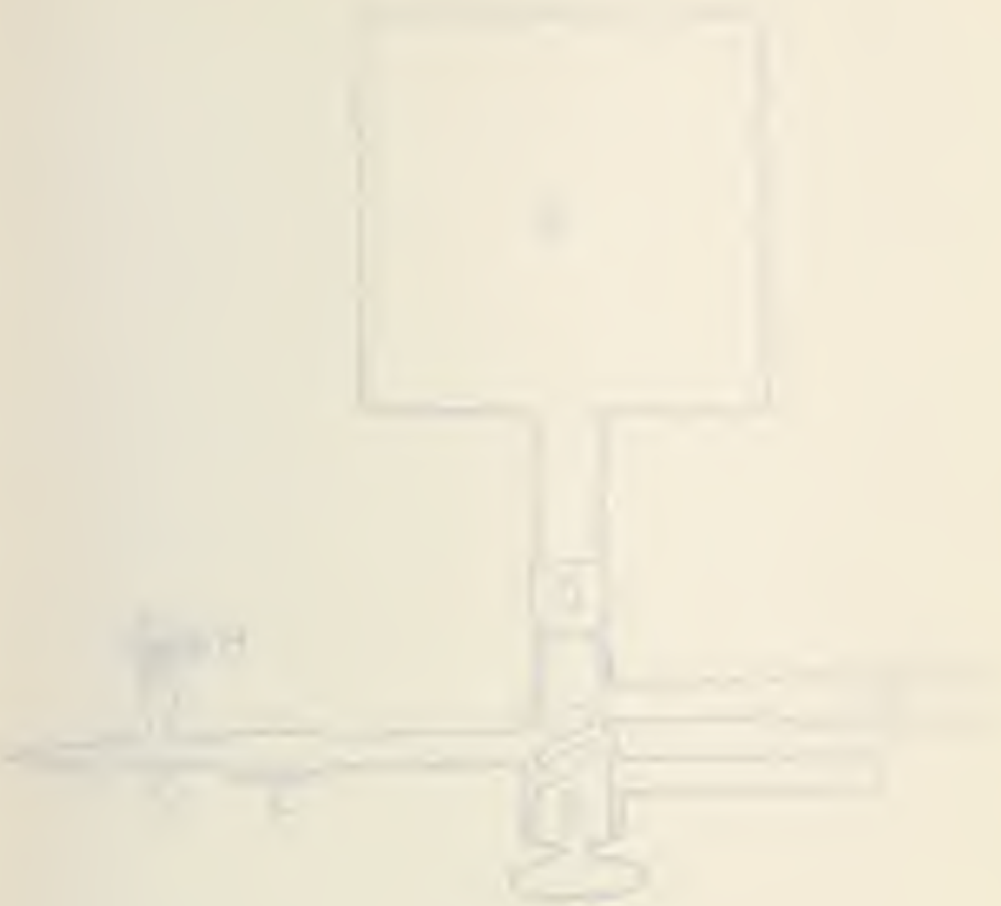
(iii) Artificial Respiration Apparatus.

When it was desirable to use high doses of drugs it became necessary to keep the animals alive by artificial respiration.

A simple means of giving artificial respiration designed for such experiments is shown in Figure 2 and in operation in Figure 1.

The butt-end of an ordinary three way glass stop-cock was attached by means of a short piece of rubber tubing to the axle of a slow-speed motor. The single outlet of the stop-cock was connected to a tracheal cannula made from a glass T-tube. A rubber tube connected the compressed air to one of the inlet tubes of the stop-cock. A three way stop-cock was used so that connection with the outlet tube was made only once per revolution of the motor.

The amount of air entering the lungs is controlled by an adjustable screw-clamp on a piece of rubber tubing attached to the side-arm of the cannula. Air is forced into the lungs only when the oblique passage in the stop-cock connects the compressed air tube to the cannula tube. During the balance of the complete revolution, expiration takes place through the side-arm of the cannula, which must always be partially open.



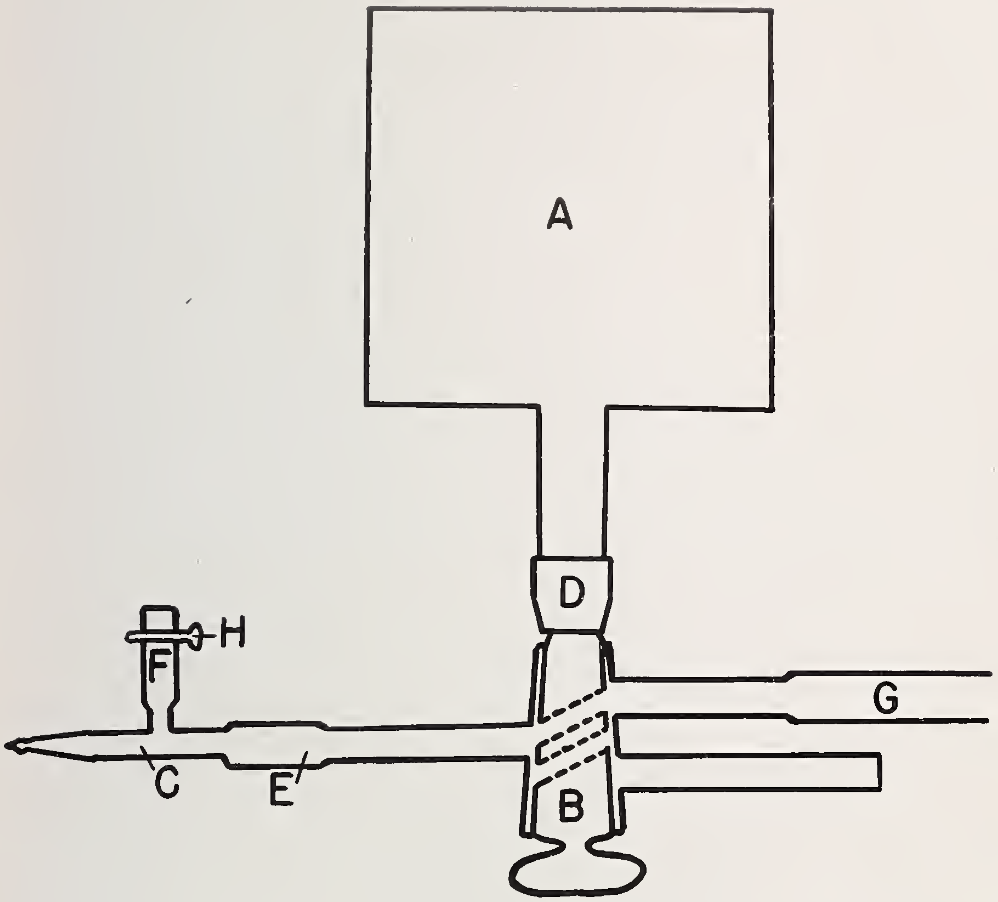
A- [illegible]TOR   D- COMPRESSED AIR

B- [illegible]   E- [illegible]

C- TRACHEAL CANNULA

F,G,H,- RUBBER CONNECTIONS

FIGURE V



A- MOTOR
B- STOPCOCK
C- TRACHEAL CANNULA
D,E,F,- RUBBER CONNECTIONS
G- COMPRESSED AIR
H- SCREW CLAMP

FIGURE 2

Efficient ventilation of the lungs was accomplished in this manner and animals which have had triple the LD50 dose of Benadryl have been kept alive for several hours.

B. PROCEDURE

The spinal cord of the rat was sectioned at the upper lumbar region under ether anesthesia and the rat left twenty-four hours to recover from the effects of the anesthetic. The animal was then tied down on the small table (Fig. 1). The calcaneous tendon was freed from the skin and fascia and severed at its attachment to the calcaneous bone. A cord attached to the tendon was secured to the isometric spring.

The sciatic nerve was exposed above the point where it enters the muscle and was clamped in a shielded electrode. It was then cut centrally. When denervated preparations were used, stimulation was by means of fine wire leads stitched into the belly of the muscle and attached to the tendon.

A normal level of response to electrical stimulation was obtained for approximately 90 minutes before a drug was administered.

The measurement of response was made from the resting level of the muscle and was calculated as force of contraction in grams. Experimental changes were reported as a percentage of the contraction tension before injection with the experimental drug.

In denervation experiments one leg was partially denervated by aseptic section of the sciatic nerve in one thigh. A period of five days was allowed for nerve degeneration. According to Cook and Gerard (42) degeneration would be complete in this time.

2. PERFUSION EXPERIMENTS ON THE GASTROCNEMIUS MUSCLE OF THE ISOLATED DOGS' LEG.

A. Apparatus (Figures 3 and 4).

A pulsating perfusion apparatus was designed and constructed for these experiments. The most important parts of such a device are the valves to control the direction of flow of the blood and a mechanism to simulate the pumping of the heart. The delivery mechanism of the Sterling Automatic Pipette, Model 4-3M (Ivan Sorvall, New York) effectively embodies both these features. The amount of perfusate delivered per stroke can be regulated from 0.5cc to 3.0cc by adjustment of the stroke regulator. In order to control the rate of pulsation, the delivery mechanism of the pipette was attached by means of a reduction gear to a variable speed motor. It was found, however, that the perfusion pressure could be adequately adjusted by means of the stroke regulator when the delivery rate is fifty strokes per minute.

Figure 3 presents diagrammatically the main features of the perfusion apparatus.

The tubing and clamps for the leg are attached to a perpendicular board A. The motor for the pump and the apparatus for the constant temperature water bath are placed behind the board. Just the head of the pipette B and a small chamber of the water bath C protrude through holes in the board. In this way the work area is kept free of apparatus. The electrical equipment is controlled by switches on plate D.

FIGURE 8

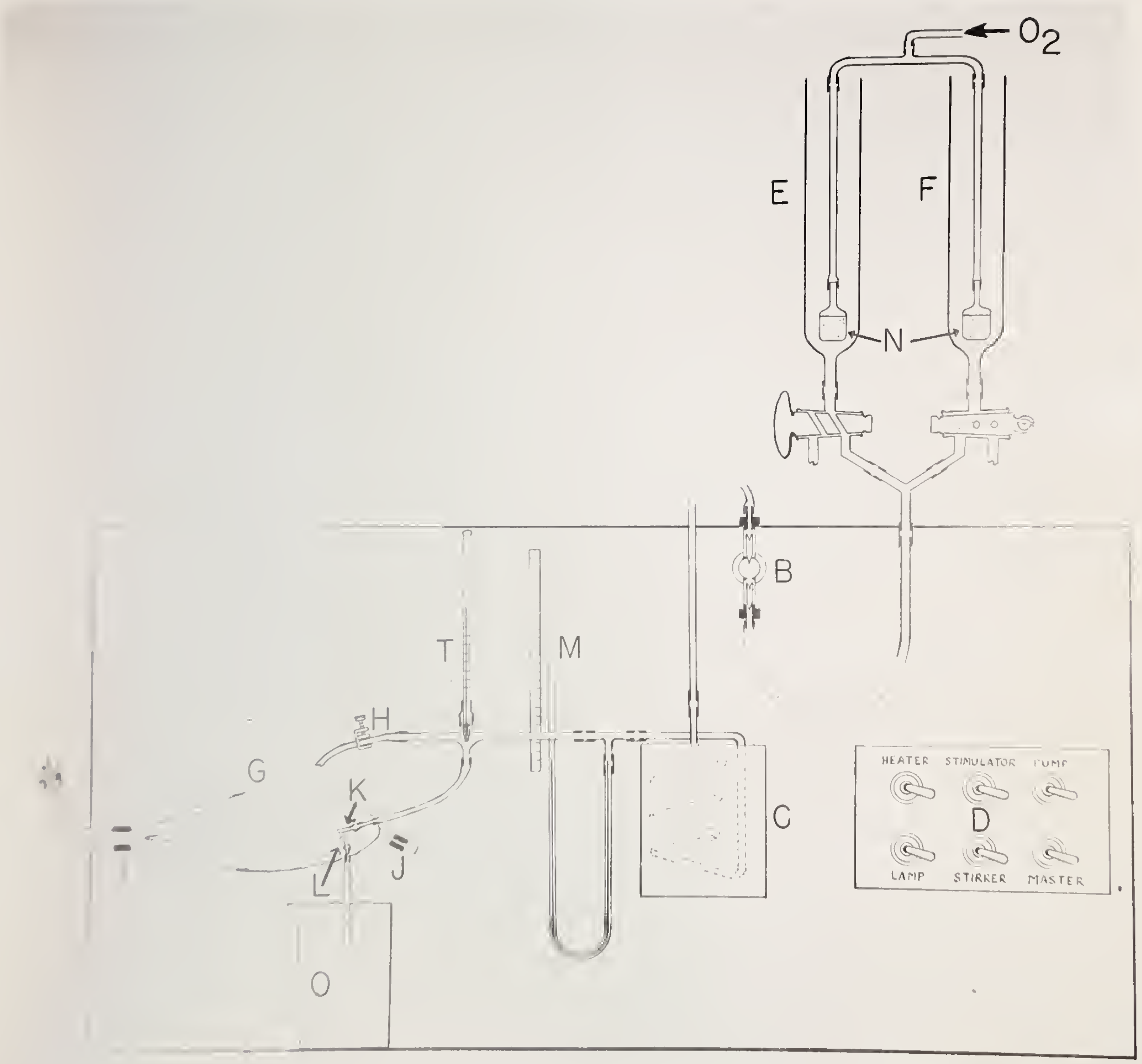

O2
E
F
N
B
T
M
H
G
K
J
L
C
HEATER
STIMULATOR
PUMP
D
LAMP
STIRRER
MASTER
O

FIGURE 3

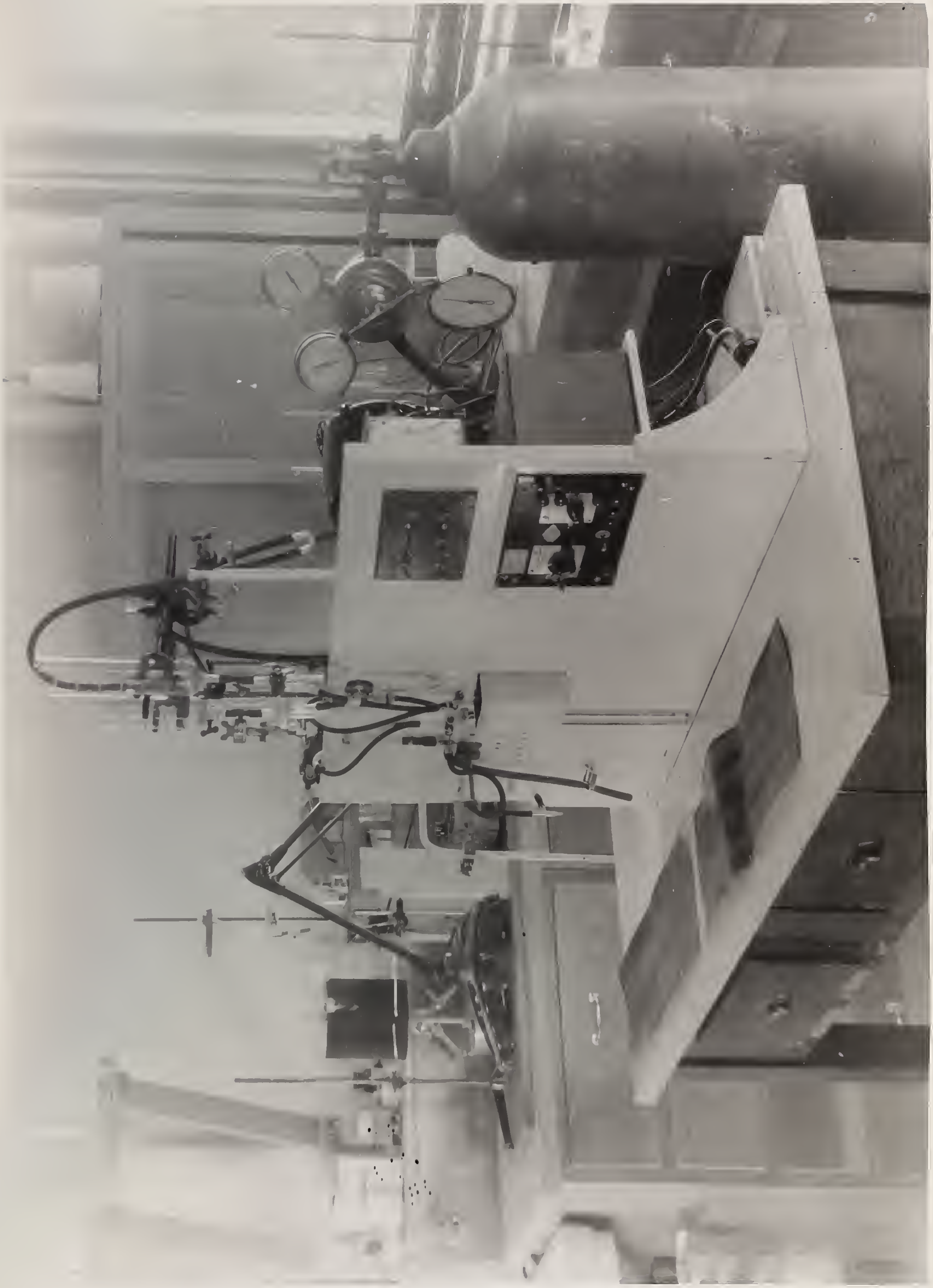


FIGURE 4

The blood is stored in two reservoirs E and F; one is for normal and one for experimental blood. Oxygenation is accomplished by bubbling oxygen through small Mandler-type filters in the reservoirs. A two way stop-cock at the bottom of each reservoir provides a by-pass.

The blood passes from the reservoir to the pump B and to the leg G through a coil in a constant temperature water bath C. The perfusion pressure is recorded on a manometer M; and the temperature on the thermometer T. H is a by-pass for rapid withdrawal of blood from the system.

Muscular contraction was initiated by shocks from an electronic stimulator.

B. Procedure.

(i) Operative Technique.

Preparation of the dogs' hind leg for amputation was carried out by the method described by Huston, Martin and Dille (43).

(ii) Perfusion Technique.

The artery was cannulated and sufficient blood pumped through the leg to wash out coagulable blood. The pump was then stopped while the vein was cannulated. Each cannulation required about one minute.

The leg was amputated, leaving sufficient of the femur for clamping, and the animal removed. Femur clamps attached to the upright board were placed at the ankle, crest of the tibia, and the femur. The latter clamp was on a rod bent at right angles to the vertical board. When the leg was fast, the

the cannulae were carefully adjusted on lead supports to insure maximum flow of blood. The sciatic nerve was placed in a shielded electrode held by a lead support. For direct stimulation of the muscle, one copper wire was sewed through the gastrocnemius at the knee and another tied around the calcaneous tendon. The thread attached to this tendon passed over a pulley wheel to a table 25cm below, where it was attached to a weak isometric spring equipped with a writing lever. The muscle was kept from drying by the application of absorbent cotton soaked in Ringer's Solution.

The perfusing blood was aerated by bubbling oxygen through small Mandler-type filters. Fresh, defibrinated blood, collected under Nembutal anaesthesia, was used throughout. This blood was heated to 37°C and was pumped at an average pressure of 150mm. of mercury.

A normal level of response to electrical stimulus was obtained before switching to the experimental blood. When a satisfactory experimental record was obtained, recovery to normal was attempted by returning to the normal blood. Usually a good recovery was obtained and was taken as one criterion of an acceptable experiment. However, in some experiments where very high doses of drugs were given to produce depression, recoveries were not always possible.

3. PERFUSION EXPERIMENTS USING THE ISOLATED RABBITS' LEG.

Perfusion of the isolated rabbit's leg did not prove successful in this investigation. Some fifteen experiments were attempted. In animals weighing in the order of 2.5Kg. cannulation

was rather difficult due to the small diameter of the blood vessels. In animals over 2.7 Kg. cannulation was usually successful although in some cases it was necessary to cannulate near the abdominal wall where the femoral vessels are larger.

When cannulation was successful and the perfusion with defibrinated blood satisfactorily begun, a vasoconstrictive action was manifest almost immediately and the perfusion rate decreased rapidly with an accompanying marked rise in the perfusion pressure. The muscle soon became so edematous that perfusion of the experimental blood was not attempted.

Several experiments were conducted to test the vasoconstrictive action of various perfusates. These perfusates were:

a. serum - previously heated to 50°C for ten minutes.
b. serum
c. suspension of washed cells in isotonic saline.
d. isotonic saline solution plus acacia 6%.
e. defibrinated whole blood and acacia 6% (50/50)
f. defibrinated whole blood
g. isotonic saline solution
h. citrated blood
i. heparinized blood

In almost every case, some degree of vasoconstriction was noted. In two experiments using defibrinated blood, the perfusion rates and pressures were satisfactory but the muscles were not maintained by the perfusates. Responses to both direct and indirect stimulation decreased rapidly to zero.

In view of the difficulties of maintaining the muscle in a satisfactory physiological state it was felt that the rabbit was unsuitable for this experimental work.

EXPERIMENTAL

2. Results.

When a drug under investigation showed an effect on the normal muscle, a series of experiments was run also on the denervated muscle in an effort to localize the point of action of the drug.

In early experiments conducted in this laboratory, the response of normal muscle was found to decrease slowly over the experimental interval due possibly to fatigue, or to temperature changes in the muscle. To establish a criterion for the experimental results, it was therefore necessary to run a series of control experiments. The response of denervated muscle also decreased over the experimental interval so a series of control experiments was run on the denervated muscle as well. These results were published in detail by Riedel and Huston (45) so are not reproduced herein, although the contraction tensions of the present investigation are calculated on the basis of these control experiments.

A. BENADRYL HCL

(i) Experiments using the Gastrocnemius muscle of the Rat.

The LD50 dose of Benadryl in the rat has been reported by Rieveschl and Grühzit (37) to be 61 mg/Kg (±9.0 S.E.) when administered by the intraperitoneal route. Preliminary experiments showed that this dosage had no effect on the muscle response. Increased dosages were employed until it was found that 150 mg/Kg would produce some effect on the muscle response. A series of experiments was conducted at this level and the results are shown in Table I. Seventy-three percent of the animals survived fifteen minutes at which time the average decrease in response was about fifteen percent. Only forty percent of the

TABLE I.

EFFECT OF BENADRYL HCL 150mg./Kg. ON THE RESPONSE OF THE NORMAL GASTROCNEMIUS MUSCLE OF THE RAT.

Figures represent percentage of initial contraction tension.

Time in Minutes.

	3	6	9	12	15	30	45	60
	94	85	78	74	65	51	29	--
	93	71	71	71	71	73	76	--
	100	97	97	92	92	76	68	68
	110	68	72	72	52	--	--	--
	104	104	107	99	99	88	85	--
	103	108	115	115	115	--	--	--
	92	96	93	98	96	--	--	--
	96	95	96	96	93	84	78	75
	90	73	59	51	48	--	--	--
	94	92	87	87	87	--	--	--
	86	90	86	89	89	89	89	89
	93	85	--	--	--	--	--	--
	98	93	--	--	--	--	--	--
	109	109	--	--	--	--	--	--
	104	100	77	--	--	--	--	--
MEAN	97.7	91.0	86.5	85.8	82.4	76.8	70.8	77.3
S.D.M.	1.8	3.3	4.6	5.3	6.3	5.8	8.8	6.1

animals survived over forty-five minutes with an average decrease in response of twenty-five percent.

Although some workers (37) attributed the death of animals from lethal doses of Benadryl to respiratory failure, others (38, 39) have suggested that death occurs from general exhaustion or from respiratory and cardiac failure. Since there was a possibility, then, that respiratory failure was the cause, it was thought of value to employ artificial respiration while recording the muscle effect of the drug. Table II shows the results on the normal muscle in experiments using artificial respiration with a dosage of 150mg/Kg. and Table III shows the results with the same dosage using the denervated muscle. In these latter experiments, seventy percent of the animals survived over sixty minutes. A comparison of the effect of this dosage on the normal and denervated muscle is shown in Table IV. In figure 5 this comparison is presented graphically.

The results on the normal muscle after sixty minutes, indicated a decrease to forty-nine percent of the normal control mean; the corresponding response of the denervated muscle was about sixty-four percent. Since both normal and denervated muscles show a definite decrease in response, it would appear that the effect is chiefly on the muscle itself rather than on the neuromuscular junction. The difference in these two values is probably not of significance since the S.D.M. value is rather high in each case.

A second comparative series was conducted on the normal and denervated muscle using artificial respiration and a dosage of 250 mg/Kg. In these experiments, ninety percent of the animals survived sixty minutes and fifty-five percent of them

TABLE II.

EFFECT OF BENADRYL HCL 150mg./Kg. ON THE RESPONSE OF THE NORMAL GASTROCNEMIUS MUSCLE OF THE RAT.

(Artificial Respiration.)

Figures represent percentage of initial contraction tension.

	Time in Minutes.								
	3	6	9	12	15	30	45	60	75
	101	104	96	88	85	80	81	76	74
	103	100	89	70	56	10	0	0	--
	99	100	101	98	95	92	89	85	85
	100	117	112	108	99	50	8	0	--
	101	116	122	118	115	30	--	--	--
	96	93	93	90	90	73	40	24	--
	98	96	95	83	66	8	--	--	--
	97	93	91	90	90	88	85	86	86
	90	67	49	27	21	--	--	--	--
	97	75	58	40	27	12	--	--	--
MEAN	98.2	96.1	90.6	81.2	74.4	43.7	50.5	45.2	81.7
S.D.M.	1.14	5.41	7.00	9.02	9.98	11.8	16.41	17.05	3.84

TABLE III.

EFFECT OF BENADRYL HCL 150mg./Kg. ON THE RESPONSE OF THE DENERVATED GASTROCNEMIUS MUSCLE OF THE RAT.

(Artificial Respiration).

Figures represent percentage of initial contraction tension.

	Time in Minutes								
	3	6	9	12	15	30	45	60	75
	95	94	91	89	87	76	71	66	60
	98	91	79	75	71	67	59	51	46
	100	97	92	90	81	70	65	53	49
	99	87	80	76	74	67	62	--	--
	98	95	80	67	62	56	49	43	36
	96	87	82	77	73	62	56	53	47
	90	86	81	78	75	71	67	--	--
	94	93	93	87	75	66	62	58	45
	95	95	89	85	78	50	42	32	15
	96	94	90	86	83	79	79	79	79
MEAN	96.1	91.9	85.6	80.9	75.8	66.2	61.2	54.4	47.1
S.D.M.	0.91	1.24	1.81	2.37	2.20	2.75	3.35	5.0	6.47

TABLE IV.

COMPARISON OF EFFECT OF BENADRYL 150mg/Kg. ON THE NORMAL AND DENERVATED GASTROCNEMIUS MUSCLE OF THE RAT.

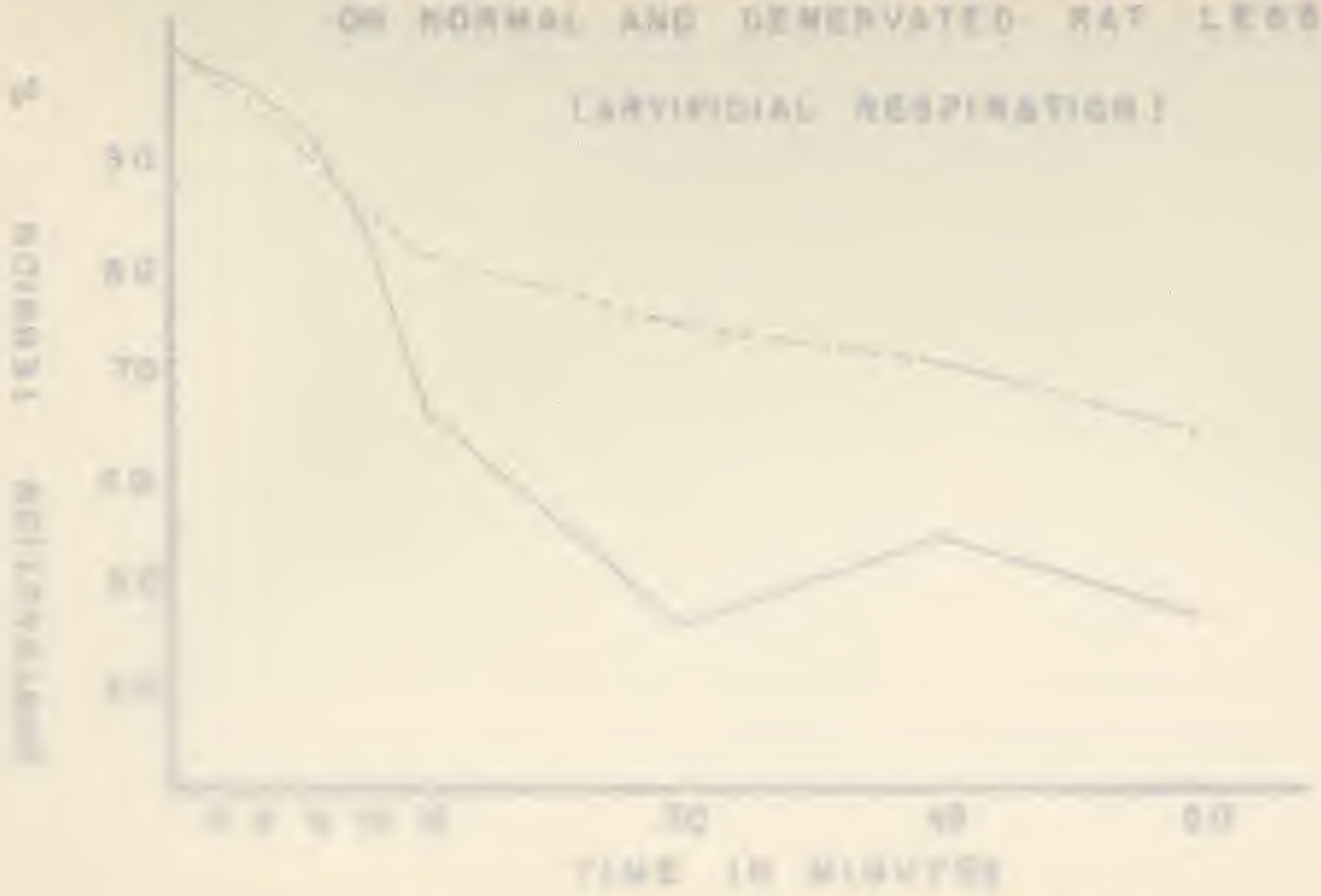
(Artificial Respiration)

Figures represent experimental mean as percentage of corresponding control mean.

Time in Minutes.

	3	6	9	12	15	30	45	60	75
Normal Muscle	97.3	95.7	91.7	82.3	75.9	45.6	53.4	48.8	89.6
Denervated Muscle	97.1	93.8	88.5	84.5	80.3	73.6	70.1	63.6	55.6

COMPARISON OF EFFECT OF BENADRYL 150 MG/KG
ON NORMAL AND DENERVATED RAT LEGS
(ARTIFICIAL RESPIRATION)



——— NORMAL LEG BASED ON CONTROL CURVE
- - - - DENERVATED LEG BASED ON CONTROL CURVE

FIGURE 8

COMPARISON OF EFFECT OF BENADRYL 150 MG/KG

ON NORMAL AND DENERVATED RAT LEGS

(ARTIFICIAL RESPIRATION)

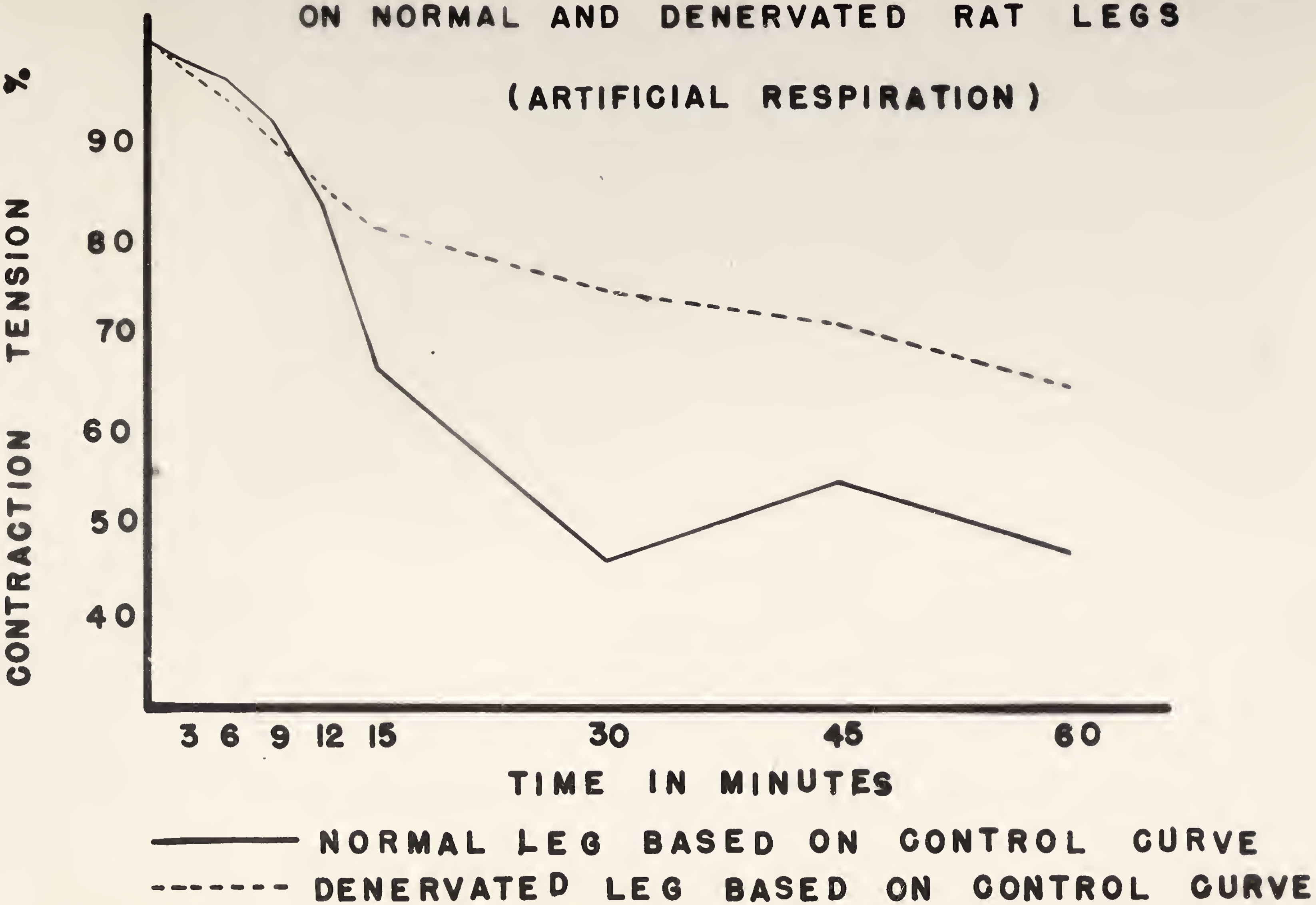


FIGURE 5

lived over 120 minutes. Table V shows the results on the normal leg; Table VI the results on the denervated leg and a comparison of the two results is presented in Table VII and in Figure 6. At this dosage, the denervated muscle shows a greater depression (to 7% of normal) than the normal muscle which shows a depression to about 29% of normal. These results provide further evidence that the effect of the drug is chiefly on the muscle and not on the neuromuscular junction mechanism. The significance of the difference in the two values is questionable since the denervated muscle, because of its altered physiological state, may be more susceptible to the high concentration of the drug.

(ii) Perfusion experiments using the isolated dogs' leg.

Since Benadryl was shown to have an effect on the gastrocnemius muscle of the rat, it was thought to be of interest to test this drug on the isolated dogs' leg. The results of the experiments conducted are summarized in Table VIII.

Two experiments using a concentration of 50 mg.% of Benadryl were run. In the first, the muscle showed a decrease to 37% of the normal in four minutes and when the drug was washed out with fresh blood, recovery of response to 72% of normal was attained in twenty-six minutes. At this time a second perfusion of the same concentration of drug was done and again a marked decrease resulted, but the degree of recovery was less pronounced. The poor recovery may be due (a) to the fact that towards the end of all perfusion experiments the muscle is in a poorer physiological condition and is therefore less able to recover from depression, or (b) to the possibility that the drug may not be completely washed out of the tissues when recovery with fresh blood is being attempted.

EFFECT OF BENADRYL HCL 250 mg./Kg. ON THE RESPONSE OF THE NORMAL GASTROCNEMIUS MUSCLE OF THE RAT.

(Artificial Respiration)

Figures represent percentage of initial contraction tension

	Time in Minutes										
	3	6	9	12	15	30	45	60	120	180	240
	79	64	64	64	62	--	--	--	--	--	--
	100	110	110	106	106	90	78	71	41	12	0
	100	100	98	95	93	79	42	19	0	0	--
	94	88	86	86	84	57	31	18	0	0	--
	95	97	93	91	89	76	62	42	0	--	--
	100	95	89	89	87	76	64	53	0	--	--
	94	89	84	81	76	49	11	0	--	--	--
	96	87	78	73	70	42	16	49	0	--	--
	99	93	84	80	74	45	9	0	--	--	--
	114	98	94	77	49	40	30	16	0	0	--
MEAN	97.1	92.1	88.0	84.2	79.0	55.4	34.3	26.8	4.1	1.2	0
S.D.M.	2.1	3.8	3.9	3.8	5.1	6.7	8.5	8.4	2.0	3.2	0

TABLE VI.

EFFECT OF BENADRYL HCL 250mg./Kg. ON THE RESPONSE OF THE DENERVATED GASTROCNEMIUS MUSCLE OF THE RAT.

(Artificial Respiration.)

Figures represent percentage of initial contraction tension.

	Time in Minutes								
	3	6	9	12	15	30	45	60	120
	98	93	86	73	70	44	13	0	--
	95	94	87	81	73	43	15	0	--
	98	94	87	77	67	17	0	--	--
	86	77	66	49	34	16	5	0	0
	90	80	75	74	72	59	10	0	--
	110	104	94	82	82	72	41	0	--
	97	84	70	58	45	35	18	0	--
	95	80	70	65	55	40	40	35	31
	94	90	79	66	62	40	23	16	0
	98	96	88	77	75	58	28	3	0
MEAN	96.1	89.2	80.2	70.2	63.5	42.4	19.3	6.0	7.8
S.D.M.	1.97	2.73	4.48	3.35	4.99	5.58	4.36	4.02	7.75

TABLE VII.

COMPARISON OF EFFECT OF BENADRYL 250mg/Kg. ON THE NORMAL AND DENERVATED GASTROCNEMIUS MUSCLE OF THE RAT.

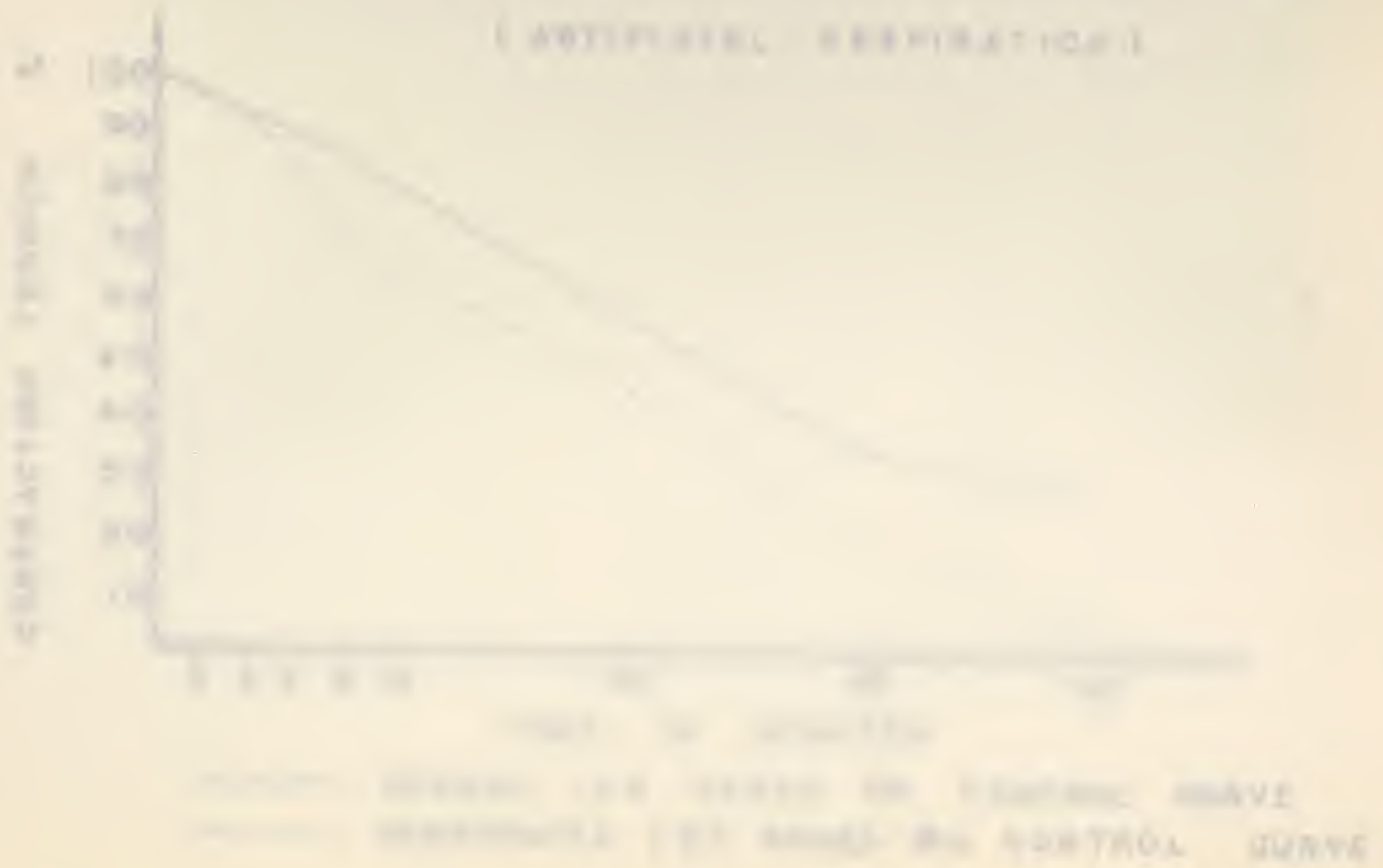
(Artificial Respiration)

Figures represent experimental mean as percentage of corresponding control mean.

Time in Minutes.

	3	6	9	12	15	30	45	60	120
Normal Muscle	96.2	91.7	89.0	85.3	80.6	57.8	36.2	28.9	4.6
Denervated Muscle	97.1	91.0	82.9	73.3	67.3	47.1	22.1	7.0	9.5

COMPARISON OF EFFECT OF BENADRYL 150 MG/KG
ON NORMAL AND DENERVATED RAT LEGS
(ARTIFICIAL RESPIRATION)



COMPARISON OF EFFECT OF BENADRYL 250 MG/KG
ON NORMAL AND DENERVATED RAT LEGS
(ARTIFICIAL RESPIRATION)

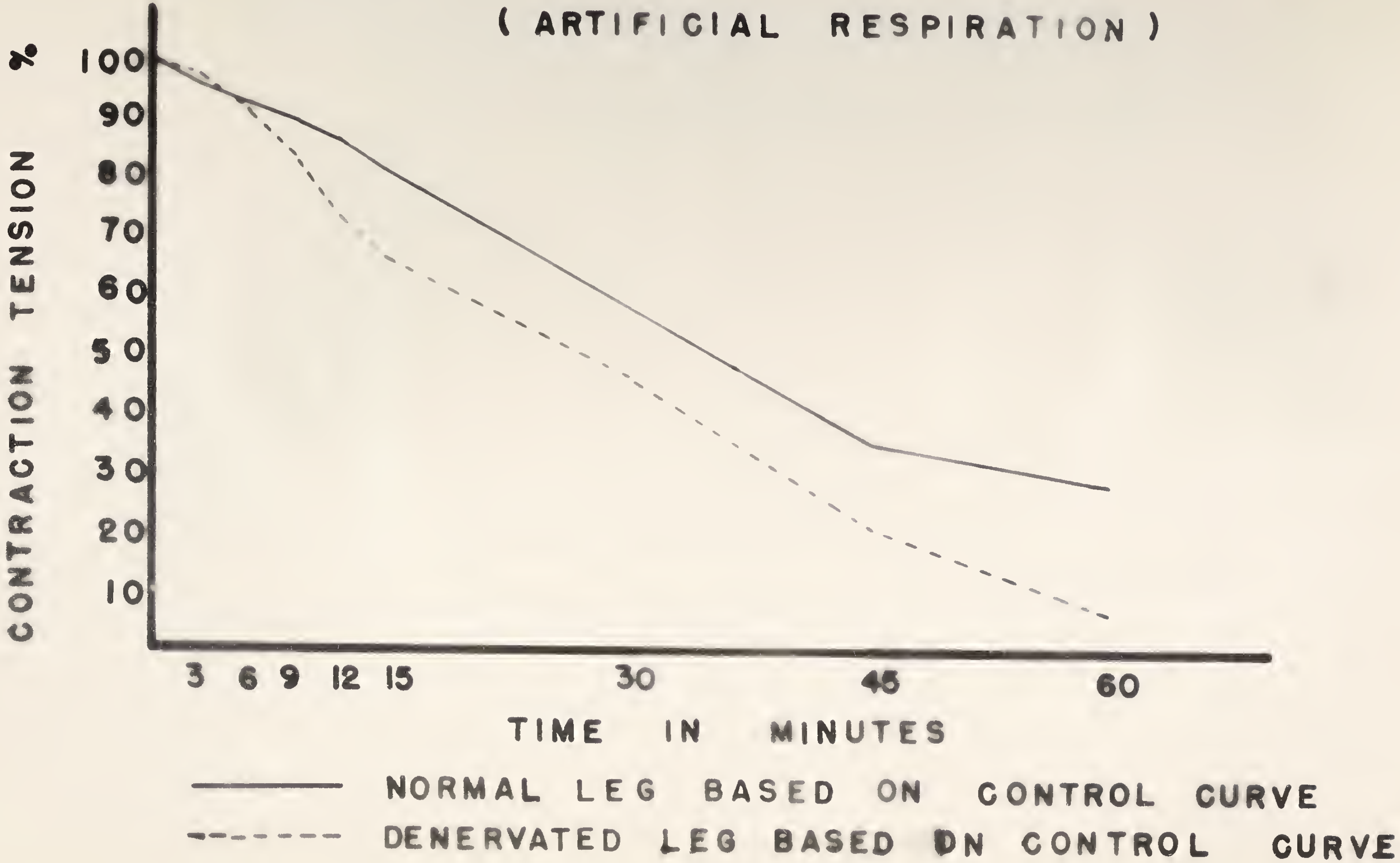


FIGURE 6

TABLE VIII.

EFFECT OF BENADRYL HCL ON THE RESPONSE OF THE ISOLATED DOGS' LEG.

NO.	LEG	DOSE (mg.%)	MAXIMUM DECREASE		MAXIMUM RECOVERY	
			TIME (MINUTES)	CONTRACTION TENSION (% OF NORMAL)	TIME (MINUTES)	CONTRACTION TENSION (% OF NORMAL)
1.	Normal	50	4	37	26	72
		#50	5	48	7	56
2.	Normal	50	6	5	11	7
3.	Normal	150	15	42	30	60
4.	Normal	150	12	8	24	65
5.	Normal	150	9	19	21	56
6.	Normal	150	4	12	9	34
7.	Normal	150	11	0	90	23
8.	Normal	150	4	29	15	57
9.	Normal	150	6	43	30	112
10.	Normal	150	3	26	66	107
11.	Normal	150	3	35	30	67
12.	Normal	150	6	17	15	33
13.	Denervated	150	9	8	150	78
		#150	11	6	33	28
14.	Denervated	150	30	0	72	38
15.	Denervated	150	4	56	22	90
		#150	15	27	21	42
16.	Denervated	150	12	37	30	45
17.	Denervated	150	6	0	30	7
18.	Denervated	150	6	33	40	86
		#150	11	11	20	22

Second dose given after maximum recovery from initial dose.

Using a dosage of 150 mg.% on the normal leg, a marked decrease in response resulted in each of the ten experiments. When the drug was washed out with new blood, the muscle response showed varying degrees of recovery. In six experiments on the denervated leg, a marked decrease again resulted. Here the figures for recovery vary also. In three of the experiments, a second dose of the drug was given but on perfusion of the fresh blood, recovery was only slight. This may be explained again by the probability that the denervated muscle may be more susceptible to the drug, the washing out process may not be complete or the general condition of the muscle after a long perfusion time may render it less responsive to the recovery process.

An interesting vasodilator action of Benadryl was noted in each perfusion. As soon as the Benadryl perfusate reached the tissues, the perfusion rate increased and the perfusion pressure dropped. The results of this observation in three experiments are shown in Table IX.

B. PYRIBENZAMINE HCL.

(i) Experiments using the gastrocnemius muscle of the rat.

The maximum dose of Pyribenzamine that could be employed without an excessive rate of mortality was 200 mg/Kg. A series of ten experiments was run at this dosage and the results are tabulated in Table X. A slight depression was produced. Further investigation of the effect of a higher dosage of this drug on the rat muscle using artificial respiration might be of interest.

TABLE IX.

EFFECT OF BENADRYL HCL 150 mg.% ON RATE AND PRESSURE IN PERFUSION OF THE ISOLATED DOG LEG.

	No. 1		No. 2		No. 3 (Denervated)	
	Rate Drops/30sec.	Pressure mm./Hg.	Rate Drops/30sec.	Pressure mm./Hg.	Rate Drops/30sec.	Pressure mm./Hg.
Defibrinated Blood	38	180	23	310	26	80
Blood with Benadryl	46	130	35	250	38	55
Recovery with Defibrinated Blood	38	200	20	270	49	80

TABLE X.

EFFECT OF PYRIBENZAMINE HCL 200mg./Kg. ON THE RESPONSE OF THE NORMAL GASTROCNEMIUS MUSCLE OF THE RAT

Figures represent percentage of initial contraction tension

	Time in Minutes						
	3	6	9	12	15	30	45
	100	100	95	95	95	91	92
	100	76	99	--	--	--	--
	100	100	96	96	96	96	96
	84	--	--	--	--	--	--
	91	89	89	89	89	89	89
	88	88	86	--	--	--	--
	92	92	92	80	80	80	82
	66	62	62	--	--	--	--
	87	87	93	84	77	74	71
	96	101	101	101	101	101	--
	92	85	85	--	--	--	--
	100	103	99	84	81	80	--
	98	94	--	--	--	--	--
MEAN	91.8	89.8	90.6	89.9	88.4	82.3	86.0
S.D.M.	2.7	3.3	3.2	2.9	3.5	4.2	4.4

(ii) Perfusion experiments using the isolated dogs' leg.

Since little effect could be shown on the gastrocnemius muscle of the rat under the experimental conditions employed, it was thought that the higher concentrations possible by the perfusion technique might show an effect. The results of these perfusions are summarized in Table XI.

In two experiments using a dosage of 50 mg.% a marked decrease resulted in three minutes. In one experiment this decrease amounted to 59% of normal with a subsequent recovery to 66% of normal while in the other test, the decrease was 34% of normal and the subsequent recovery was to 52% of normal.

In two experiments at a dosage of 100 mg.% the resultant decrease was pronounced and the extent of recovery was appreciable.

When the concentration of drug was increased to 150 mg.%, the muscle response decreased to 34% of normal in one case, and to zero in the other test. Since no recovery was obtained after perfusion of fresh blood, there is a possibility that the drug at this concentration produces an irreversible effect on the muscle.

Two denervation experiments were run. In the first, a dosage of 50 mg.% caused an increase to 121% within one minute. This response at nine minutes was still 110% of normal. An addition of 50 mg. of Pyribenzamine was made to the perfusate at this time and within twenty-four minutes the response had dropped to normal. In the second experiment, 50 mg.% again produced an increase in response to 115% of normal in one minute.

TABLE XI.

EFFECT OF PYRIBENZAMINE HCL ON THE RESPONSE OF THE ISOLATED DOGS' LEG.

No.	LEG	DOSE (mg.%)	MAXIMUM EFFECT		MAXIMUM RECOVERY	
			TIME (MINUTES)	CONTRACTION TENSION (% OF NORMAL)	TIME (MINUTES)	CONTRACTION TENSION (% OF NORMAL)
1	Normal	50	3	59	6	66
2	Normal	50	3	34	12	52
3	Normal	100	6	4	17	62
4	Normal	100	2	23	9	42
5	Normal	150	6	34	--	--
6	Normal	150	6	0	--	--
7	Denervated	50	1	121	9	110
		100	10	109	24	100
8	Denervated	50	1	115	5	107
		150	15	70	25	85

After five minutes, the response was still 107% of normal and 100 mg. of drug was added to the perfusate. The response dropped to 70% of normal and when fresh blood was perfused, the recovery reached 85% of normal. There is a possibility that the drug in a low concentration has a depressant action on the neuromuscular junction and a stimulant action on the muscle, while a high concentration may have a depressant action on both the motor end plate area and the muscle.

C. CAMPHOR

According to Molitor (46), the intraperitoneal administration of 600 mg/Kg. of camphor will cause convulsions without being fatal. Preliminary experiments in the present investigation showed no peripheral effect at this concentration. When a dosage of 1200 mg/Kg. was administered, the animals died in an average time of fifteen minutes. When artificial respiration was employed however, life was prolonged sometimes several hours.

In all camphor experiments, a 25% solution of the drug in sesame oil was used. Intraperitoneal injection was the route of administration in each case. Table XII shows the results of ten experiments on the normal muscle using camphor 1200 mg/Kg. and employing artificial respiration. Eighty percent of the animals lived over one hour and fifty percent lived over three hours. The drug produced a marked depression in the muscle response.

Table XIII shows the results of a similar series using the denervated muscle. Although only six experiments were done, it would appear that the drug causes some depression of the response.

TABLE XII.
EFFECT OF CAMPHOR 1200 mg./Kg. ON THE RESPONSE OF THE NORMAL GASTROCNEMIUS MUSCLE OF THE RAT.

(Artificial Respiration)
Figures represent percentage of initial contraction tension.

	Time in Minutes.									
	3	6	9	12	15	30	45	60	120	180
	90	86	84	83	63	29	--	--	--	--
	92	90	85	72	62	60	--	--	--	--
	98	96	86	85	78	49	44	44	30	17
	96	93	93	87	79	54	52	52	53	--
	98	96	96	87	81	49	46	42	26	23
	100	98	97	97	94	86	76	72	57	53
	100	97	94	91	91	77	65	59	43	47
	96	96	96	93	93	74	60	59	52	53
	95	91	87	81	81	84	78	73	--	--
	96	96	90	85	85	85	53	43	--	--
MEAN	96.1	93.3	90.3	86.1	80.7	64.7	59.3	55.5	43.5	38.6
S.D.M.	1.01	1.24	1.68	2.18	3.53	6.13	4.63	4.40	5.27	7.73

TABLE XIII.

EFFECT OF CAMPHOR 1200mg./Kg. ON THE RESPONSE OF THE DENERVATED GASTROCNEMIUS MUSCLE OF THE RAT.

(Artificial Respiration)

Figures represent percentage of initial contraction tension.

	Time in Minutes.								
	3	6	9	12	15	30	45	60	120
	102	104	102	102	100	91	98	93	81
	99	99	96	92	92	82	84	81	--
	95	96	83	84	81	83	35	--	--
	96	93	91	93	89	71	--	--	--
	99	96	96	95	88	94	92	84	78
	100	98	98	85	76	37	--	--	--
MEAN	98.5	97.7	94.3	92.0	87.7	76.3	77.2	86.0	79.5
S.D.M.	1.06	1.52	2.69	2.73	3.45	8.52	14.3	3.60	1.5

Table XIV and figure 7 show a comparison of the effect of this dosage of camphor on the normal and denervated muscle. Since the normal muscle response is depressed to about 60% of normal in one hour and since there is little effect on the denervated muscle response at this time, it would appear that camphor at this dosage has a depressant effect on the neuromuscular junction but has little effect on the muscle itself. This observation is worthy of further investigation.

D. CAFFEINE

Kiskalt (47) places the fatal intraperitoneal dose of caffeine in the rat at 210-280 mg/Kg.

Using a dosage of 175 mg/Kg. on the normal muscle, a series of fourteen experiments was run. The results are shown in Table XV. A somewhat smaller series was run at the same dosage on the denervated muscle. These results are presented in Table XVI. The results of the two series are compared in Table XVII and in figure 8. It would appear that caffeine at this dosage exhibits a stimulant effect on the neuromuscular junction as well as on the muscle itself, since the response is higher in the normal muscle than in the denervated muscle. There is the possibility however, that the altered physiological state of the denervated muscle would render the muscle less responsive to the drug. It might be of value to further examine this possible neuromuscular effect by use of curarized muscle.

E. NIKETHAMIDE.

Using Nikethamide on the normal rat muscle at a dosage of 400 mg/Kg. nc effect on the muscle response was seen. The results of this series are presented in Table XVIII. Although

TABLE XIV.

COMPARISON OF EFFECT OF CAMPHOR 1200mg/Kg. ON THE NORMAL AND DENERVATED GASTROCNEMIUS MUSCLE OF THE RAT.

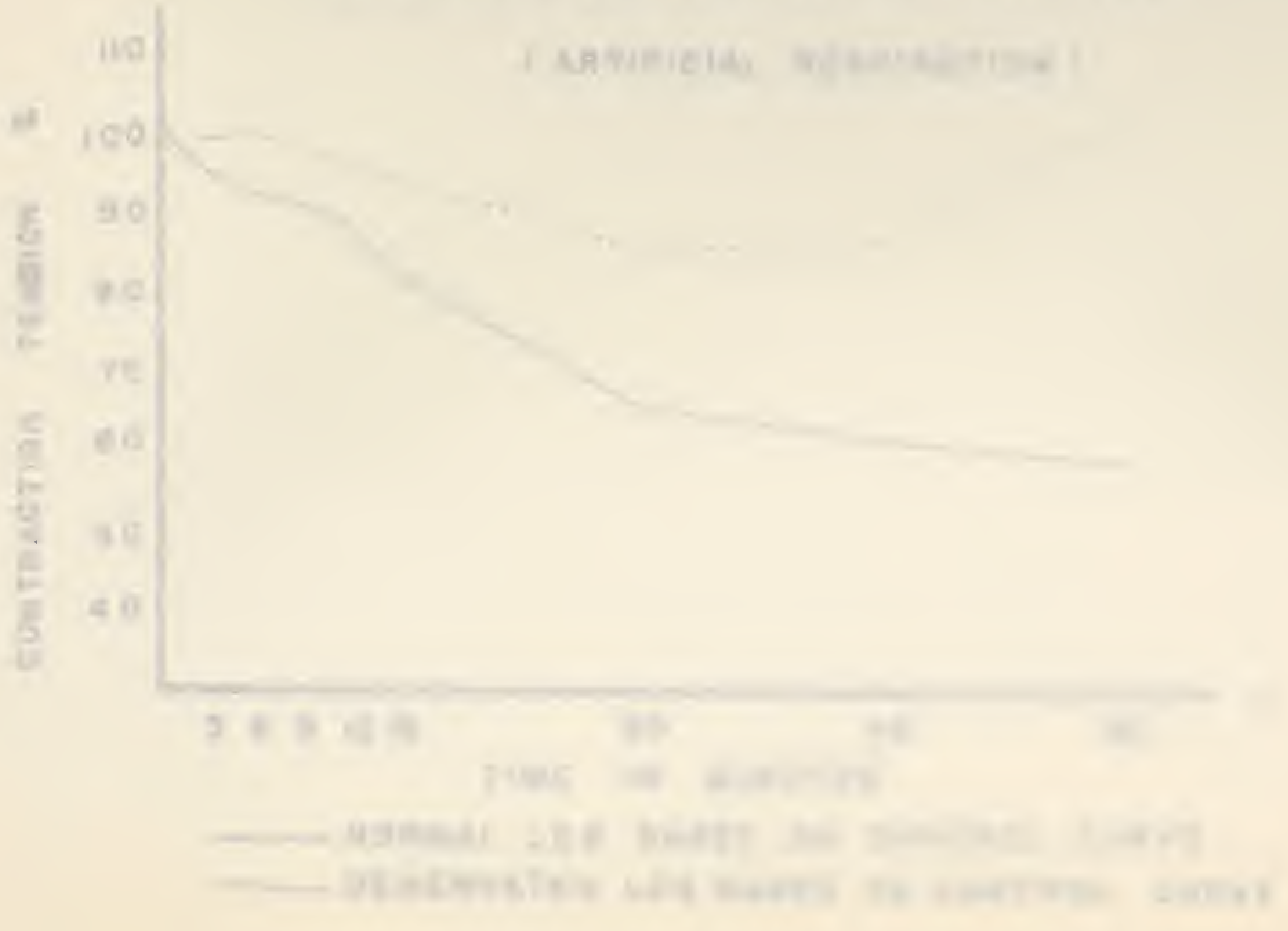
(Artificial Respiration)

Figures represent experimental mean as percentage of corresponding control mean.

Time in Minutes.

	3	6	9	12	15	30	45	60	120
Normal Muscle	95.2	92.9	91.3	87.3	82.4	67.5	62.6	59.8	49.2
Denervated Muscle	99.5	99.7	97.5	96.0	92.9	84.8	88.4	100.5	96.6

COMPARISON OF EFFECT OF CAMPHOR (200 MG/KG
ON NORMAL AND DENERVATED RAT LEGS
(ARTIFICIAL RESPIRATION)



COMPARISON OF EFFECT OF CAMPHOR 1200 MG/KG
ON NORMAL AND DENERVATED RAT LEGS
(ARTIFICIAL RESPIRATION)

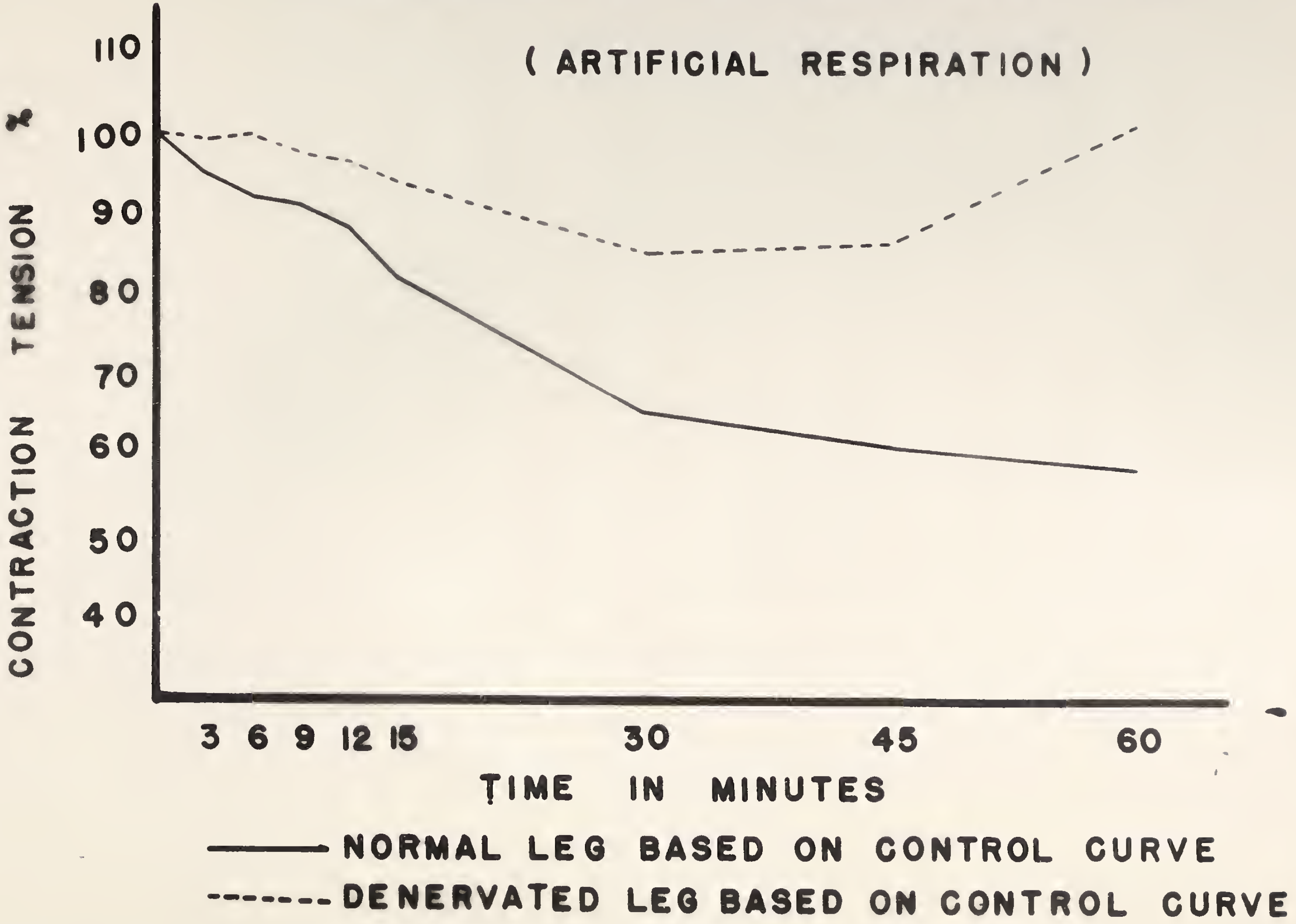


FIGURE 7

TABLE XV.

EFFECT OF CAFFEINE 175mg/Kg. ON THE RESPONSE OF THE NORMAL GASTROCNEMIUS MUSCLE OF THE RAT.

Figures represent percentage of initial contraction tension.

	Time in Minutes.							
	3	6	9	12	15	30	45	60
	165	160	150	137	137	122	121	121
	121	134	151	143	145	133	120	125
	96	121	111	111	111	112	111	115
	136	144	156	177	177	186	183	188
	106	110	115	118	122	116	116	124
	133	151	148	154	155	155	148	143
	118	132	132	125	120	86	80	77
	137	141	98	110	85	78	74	68
	125	139	115	112	112	100	78	78
	112	116	116	116	114	116	116	116
	112	114	112	106	108	98	97	93
	116	124	126	122	120	118	108	104
	116	126	122	116	113	94	92	92
	104	110	110	109	109	102	91	89
EAN	121.2	125.3	125.8	125.4	123.4	115.4	109.6	109.5
.D.M.	4.65	4.36	4.94	5.26	6.17	7.54	7.82	8.36

TABLE XVI.

EFFECT OF CAFFEINE 175mg./Kg. ON THE RESPONSE OF THE DENERVATED GASTROCNEMIUS MUSCLE OF THE RAT.

Figures represent percentage of initial contraction tension.

	Time in Minutes.							
	3	6	9	12	15	30	45	60
	97	102	104	102	101	99	97	96
	99	106	103	105	101	100	102	100
	91	99	99	103	104	88	90	86
	109	116	116	112	111	107	104	104
	99	101	101	95	93	92	94	97
	109	110	106	104	104	101	100	104
MEAN	100.7	105.0	104.8	103.5	102.3	97.8	97.8	97.8
S.D.M.	2.90	2.63	2.44	2.23	2.39	2.77	2.14	2.46

TABLE XVII.

COMPARISON OF EFFECT OF CAFFEINE 175mg./Kg. ON THE NORMAL AND DENERVATED GASTROCNEMIUS MUSCLE OF THE RAT.

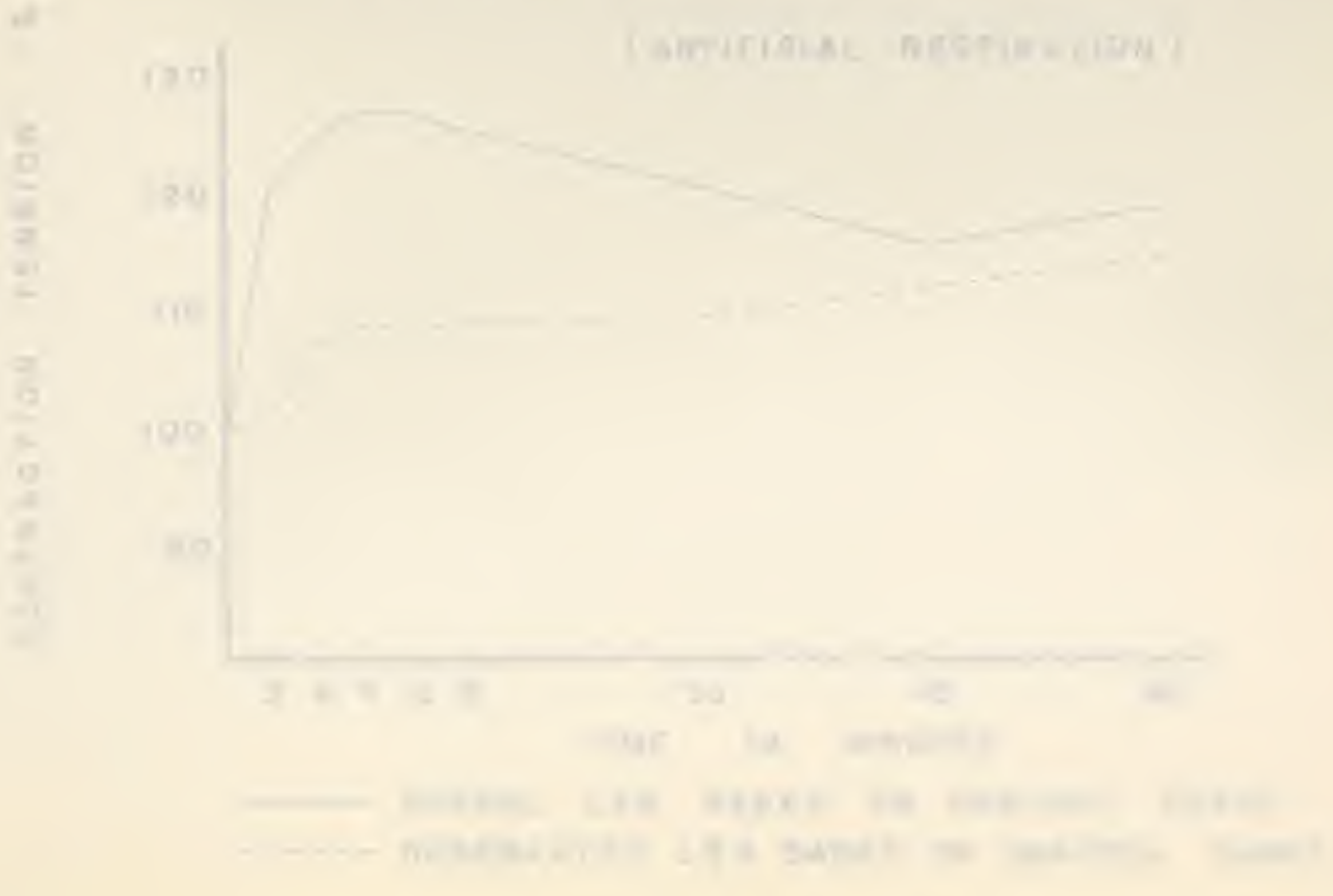
Figures represent experimental mean as percentage of corresponding control mean.

	Time in Minutes.							
	3	6	9	12	15	30	45	60
Normal Muscle	120.1	124.8	127.3	127.2	126.0	120.4	115.8	118.1
Denervated Muscle	101.7	107.1	108.4	108.0	108.4	108.7	112.0	114.3

COMPARISON OF EFFECT OF CAFFEINE (25MG/KG)

ON NORMAL AND DENERVATED RAT LEGS

(ARTIFICIAL RESPIRATION)



COMPARISON OF EFFECT OF CAFFEINE 175 MG/KG

ON NORMAL AND DENERVATED RAT LEGS

(ARTIFICIAL RESPIRATION)

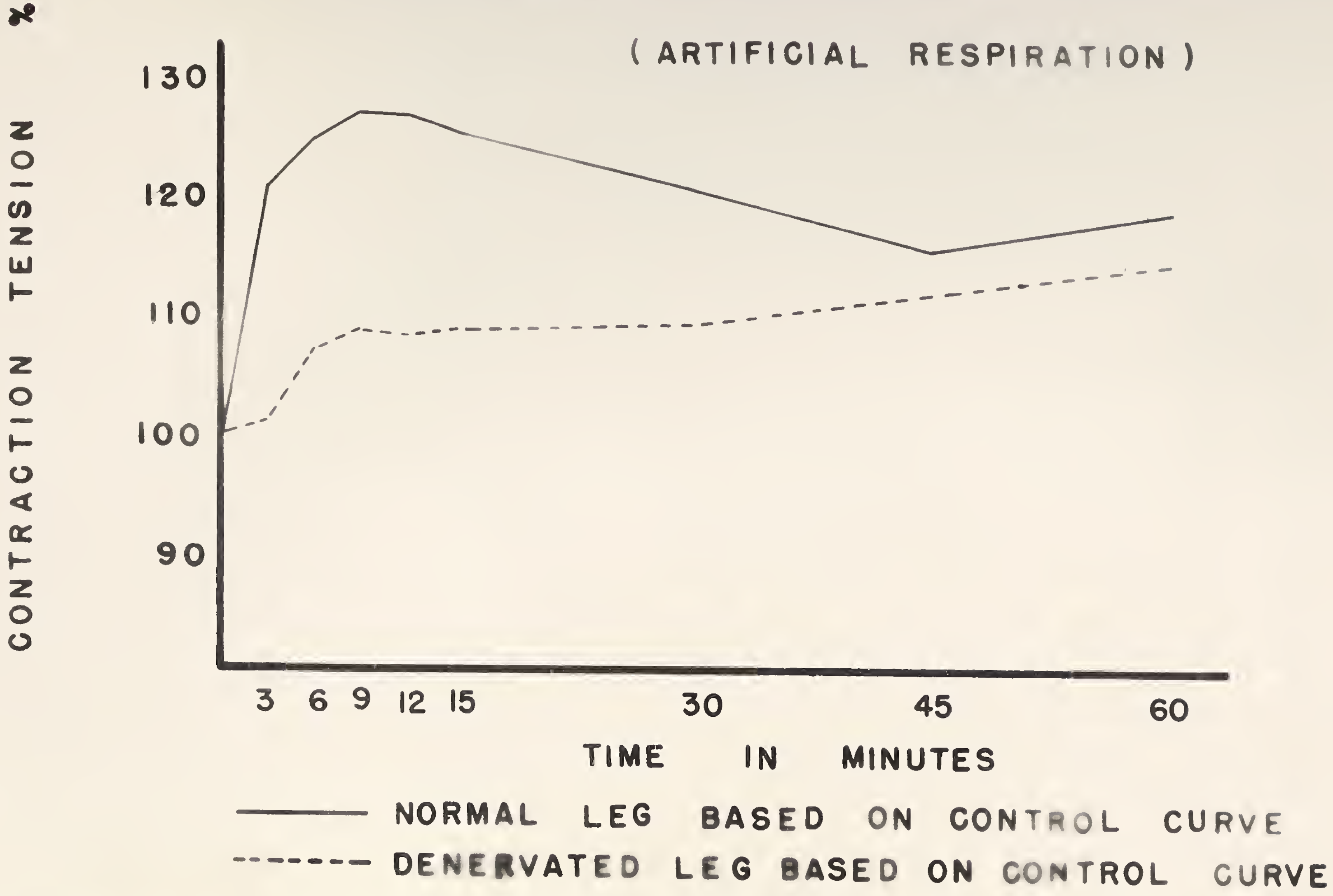


FIGURE 8

TABLE XVIII.

EFFECT OF NIKETHAMIDE 400mg/Kg ON THE RESPONSE OF THE NORMAL GASTROCNEMIUS MUSCLE OF THE RAT.

Figures represent percentage of initial contraction tension.

	Time in Minutes.								
	3	6	9	12	15	30	45	60	75
	100	94	94	94	94	--	--	--	--
	100	96	96	93	88	78	--	--	--
	99	94	92	94	88	75	--	--	--
	91	82	82	75	75	75	--	--	--
	96	100	92	96	96	100	84	76	76
	100	88	100	100	94	100	--	--	--
	103	104	104	104	104	--	--	--	--
	107	103	105	105	113	110	105	100	98
	103	103	109	105	105	126	122	--	--
	106	103	103	106	106	--	--	--	--
MEAN	100.5	96.7	97.7	97.2	96.3	94.9	103.7	88.0	87.0
S.D.M.	1.49	2.33	2.55	2.95	3.51	7.53	9.51	9.80	8.98

the fatal dose of this drug is reported as being above 450 mg/Kg., (48), only 30% of the animals lived longer than forty-five minutes.

DISCUSSION.

A. BENADRYL

Since it was possible by the use of artificial respiration to prolong the life of the rat in the experiments where high dosages of Benadryl were used, it would seem that the primary cause of death from lethal doses of the drug is respiratory failure. Although death has been attributed to general exhaustion (38) or to respiratory and cardiac failure (39) the results of this present investigation may indicate that conditions other than that of respiratory involvement are secondary, since some of the test animals were still living three hours after administration of the drug. In no case, however, did the animal recover consciousness, nor was there any recovery of muscle response. This observation would lead to the conclusion that cardiac involvement, although delayed, may be a secondary factor in causing death.

On the basis of the results herein reported, it seems reasonable to conclude that in the dosages used, Benadryl depresses the muscle response, by a direct action on the muscle itself. Since there seemed to be no significant difference between its effect on normal and on denervated muscle, there is no evidence here that Benadryl acts on the neuromuscular junction.

Friedlaender and Feinberg (48) reported a slight depression in blood pressure in humans on the administration of Benadryl and suggested that this depression may have been central in origin. In the present investigation using the isolated leg, Benadryl caused an increase in the perfusion rate while the perfusion pressure dropped. It would appear that the drug in this instance had a direct vasodilatation action.

B. PYRIBENZAMINE

Pyribenzamine in the dosage used showed little effect on the normal rat muscle. The results of two perfusion experiments on denervated muscle using 50 mg.% produced an increase in response. Since the same dosage, and higher dosages produced a definite depression in the muscle response of the normal isolated leg, there is a possibility that the drug in low dosage may have a depressant action on the myoneural junction and a stimulant action on the skeletal muscle while a high concentration may depress both the neuromuscular junction and the muscle itself. Such a possibility is deserving of further investigation.

C. CAMPHOR

The results obtained using Camphor are,in part, similar to those observed by Huston (44). He found in perfusion experiments that while a concentration of 200 mg.% of Camphor in the perfusing blood stimulated the muscle and myoneural junction, a concentration of 400 mg.% depressed the myoneural junction very markedly but the muscle only slightly. In the present rat experiments, no stimulant action was shown, but since the normal muscle response was depressed to 60% of the normal control and since there was little effect on the denervated muscle response, it would appear that the results substantiate those of Huston where he used the 400 mg.% concentration of drug.

D. CAFFEINE

Huidobro and Amenabar (49) showed that caffeine enhances the transmission of the nerve impulse in the neuromuscular junction. Huston (44) also showed a stimulant action of the drug at this site, as well as on the muscle itself.

The results in figure 8 would appear to be similar to those obtained by these workers.

E. NIKETHAMIDE

The one series of experiments done with this drug showed no effect on the response of the normal rat muscle. Further investigation might be of interest since, by the use of different experimental methods, Huidobro and Jordan (50) were able to demonstrate definite effects of Nikethamide on neuromuscular conductivity.

SUMMARY AND CONCLUSIONS

A. BENADRYL HCL.

(i) Benadryl HCL, 150 mg./Kg. injected intraperitoneally into rats caused a decrease in the response of the normal and denervated gastrocnemius muscle to electrical stimulation. At a dose level of 250 mg./Kg. a more pronounced depression was produced. This depressant action would appear to be directly on the muscle.

(ii) Benadryl HCL, 50 mg.% added to defibrinated blood being perfused through isolated dog legs caused a rapid and marked depression of the response of normal muscle. A concentration of 150 mg.% depressed the response of normal and denervated muscle. The depression would therefore appear to be due to an action of the drug on the muscle fibres.

B. PYRIBENZAMINE HCL.

(i) Pyribenzamine HCL 200 mg./Kg. injected intraperitoneally produced no significant effect on the normal gastrocnemius muscle of the rat.

(ii) In preliminary tests with Pyribenzamine HCL on the perfused dog leg the following results were obtained: a depression of response of the normal muscle with 50 mg.%; an increased response of denervated muscle with 50 mg.%; a depression of both normal and denervated muscle with 100 mg.% and with 150 mg.%.

C. CAMPHOR.

Camphor 1200 mg./Kg. injected intraperitoneally into the rat caused a depression in the response of the normal muscle but had little effect on the response of the denervated muscle. Such a result indicates a depressant action on the neuromuscular junction with little effect on the muscle itself.

D. CAFFEINE.

Caffeine 175 mg./Kg. injected intraperitoneally into the rat caused an increase of response which appears to be due to an action on both the neuromuscular junction and the muscle fibres.

E. NIKETHAMIDE.

Nikethamide 400 mg./Kg. produced no significant effect on the normal gastrocnemius muscle of the rat.

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